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ON THE QUANTITATIVE DETERMINATION OF PHENOLS SEPARATED BY THIN-LAYER CHROMATOGRAPHY*

By

Helmut Seeboth and Hedda Görsch

Work on the separation of phenols through thin-layer chromatography has been conducted on several occasions /1/. Some time ago we were able to report /2/ that simple phenols could easily be separated when we used **Super** Gel or Silica Gel A of the "VEB Farbenfabriken Wolfen", or a mixture of one of these gels with alumimum oxide "sour" of the "VEB Chemiewerk Greiz-Doelau".

Several methods can be used for the quantitative evaluation of phenols separated by thin-layer chromatography. The concentration of the phenols can be determined approximately from the size of the spot, as was shown by Patuska and Petrowitz /1/ with naphtols. Others eluted the phenols from the adsorption layer and determined their concentration by conducting absorption measurements in the ultra violet region /3/. In our laboratory we used colorimetric procedures.

First, we had intended to elute and colorimetrically determine the phenols that were made visible as azo colors through coupling with p-nitrobenzenediazoniumfluoborate. Gasparic and Beranova /4/ had already coupled phenols, separated by paper chromatography, with diazotized 2-chloro-4-nitroaniline, eluted the occurring colors, and

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* Report from the Institute of Organic Chemistry of the German Academy of Sciences in Berlin (Director: Prof. Dr. A. Riecke).

determined them colorimetrically by the Lange-Colorimeter. In our case this procedure was not possible because the phenols reacted with the diazotized amine only at the surface of the adsorption layer, and the dye formed could not be eluted completely.

Thus, there remained only the possibility of lixiviating the phenols themselves from the adsorption layer, for which water, or better yet, methanol was suitable. If water was used, it might easily have been possible that some silica gel colloid was dissolved and precipitated in the filtrate when acid was added. Of special importance was the fact that during the running time of the phenols on the plate there were no phenol losses due to evaporation.

Qualitatively we were still able to determine 5 to 19 µg of phenol /2/; for a quantitative determination the phenol concentration must be higher. This was accomplished with a somewhat thicker adsorption layer as well as by applying the substance closely together at several starting points. A chromatogram was then obtained, such as was shown previously /2/. Since the R_f-values differed somewhat on the different glass plates, and especially with different silica gel charges, it was necessary to have test mixtures run on both sides of the phenols to be tested. These test mixtures were to be made visible in the usual way, of course, by covering the rest of the glass plate simultaneously. In this way the area reserved for each individual phenol was properly defined. The layer containing the phenol to be tested was scraped off the glass plate and eluted with the solvent on a frit. The solution then underwent a coupling with p-nitrobenzenediazoniumfluoborate, and the dye formed was colorimetrically analyzed.

A prerequisite for the quantitative evaluation of a chromatogram is, of course, a good separation of the phenols. The type of separation can be determined based on the test mixtures used. Once again we shall point out here that for separating phenols, the degree of suitability of the Wolfen silica gel charges is not the same in every case. The following phenols were tested for their quantitative determinability: phenol, resorcinol, catechol and hydroquinone.

The tests were conducted with the Lange-Univerral-Colorimeter according to the deflection method. For obtaining the factors for quantitative determination, we first investigated the watery solutions of these phenols colorimetrically and in this way determined the extinction zone, the smallest determinable concentration, and the filter that should be used during the measurement. The calibration curves obtained in this manner (except those for hydroquinone) were practically identical. This was true for the calibration curves obtained after application and elution (without running) as well as with those obtained after a running time of 60 minutes and subsequent elution. The individual values fluctuated considerably. That is why the individual steps were performed in defined time intervals, especially since the extinction values of the dye solutions changed slowly while standing. Because of this the fluctuation of the extinction values was less and good calibration curves were obtained.

When a green filter is used (German Trade Designation VG9), resorcinol is well determinable in amounts between 20 and 100 µg in the extinction zone of 0.1 to 0.4. The color spot of the resorcinol on

a super gel plate is yellow-orange after it is sprayed with p-nitrobenzenediazoniumfluoborate in acetone. The buffered, watery, methanolic solution has the same color. At low concentration the alkali solution to be measured is blue-gray; as the concentration increases it turns more and more purple.

When a dark blue filter was employed (German Trade Designation BG5), hydroquinone produced a usable calibration curve for concentrations of between 100 to 500 µg in the extinction zone 0.1 to 0.4. Here, however, the absorption scale of the Lange Colorimeter could only be utilized between 0 and 90. The buffered methanolic solution of the azo dye from hydroquinone and p-nitrobenzenediazoniumfluoborate was yellow, the alkali solution red. Even though hydroquinone was quantitatively extractable after it was applied (and let run) on the super gel plate with methanol, it was not possible to set up a calibration curve after the usual coupling. Presumably, it was oxidized on the adsorption layer-perhaps even under the catalytic influence of the adsorption layer. Thus it was possible, shortly after the application, to detect p-quinone. We found that heating a super gel plate for 10 hours to 50°C in an oxygen-free stream of nitrogen did not eliminate the oxidation prior to the application of hydroquinone.

When a green filter was used, catechol could be determined in amounts of between 200 and 500 µg in the extinction zone 0.1 to 0.4. Plotting of the calibration curve presented certain difficulties here. This was due to a relatively large fluctuation of the values. Perhaps this was also connected with the oxidation sensitivity of the bivalent

phenol. But here, too, it was possible to obtain usable and almost identical calibration curves, regardless of whether the phenol was determined directly or after application upon the adsorption layer and a 60 minute running time. The color of catechol with p-nitrobenzenediazoniumfluoborate on super gel was brownish-violet. In an alkaline solution at a low concentration it was yellowish-brown; and as the concentration increased it turned dark green.

When we used a dark blue filter, phenol was measured in quantities of between 20 and 200 μg in the extinction zone 0.1 to 0.4. The calibration curve was easy to reproduce. The color spot of the phenol with p-nitrobenzenediazoniumfluoborate on super gel was yellow. The alkaline solution was light orange to light red.

These investigations have shown that the quantitative evaluation of phenols separated through thin-layer chromatography is possible by using the colorimetric method. Unfortunately, this does not hold true for hydroquinone; therefore, this method can find only limited application in low-temperature water analysis in spite of its many advantages. Nevertheless, we feel it desirable, in connection with thin-layer chromatographic separation of phenols to clarify the question as to whether or not a mutual influence takes place which makes more difficult or even impossible a quantitative determination. The tests were performed with a mixture of phenol, catechol and resorcinol with a quantitative relationship of 1 : 5 : 1. Here we found practically the same calibration curve for every phenol that the individual components had yielded (Fig. 1). As mentioned, the hydroquinone curve was obtained only upon direct application of a watery solution.

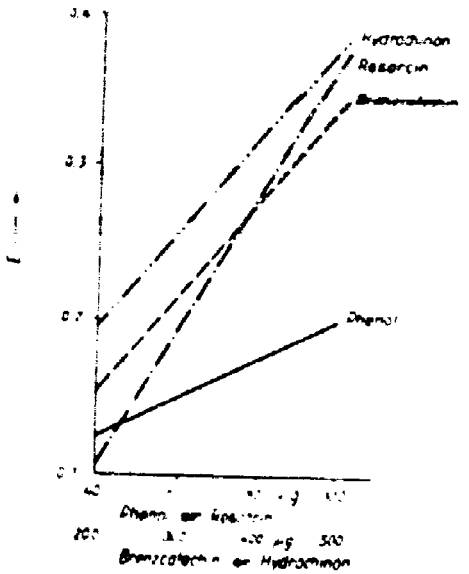

Figure 1.

Description of the Method of Analysis.

The phenols are applied in a watery solution with a pipette. The maximum applied is 0.01 cm^3 (volume of the pipette). Application of this quantity is made 2.5 cm from the narrow side and 2 cm from the long side at approximately 10 points, over an area of 5 to 6 cm. After drying, it is permissable for the starting points to just touch each other. A mixture of the phenols (test-phenols) to be determined is applied at both edges of the plate and at the same starting height. It is chromatographed in the desiccator with a mixture of benzol and glacial acetic acid (ratio 5 : 1). In order to obtain a wide separation of the phenols one should let the mixture cover as large a distance as possible. With our plates (10 X 20 cm) this distance is 16 to 18 cm at a running time of about 50 minutes.

After the plates are removed from the desiccator and the solvent is evaporated, the phenol test-mixtures that were run on the edges are sprayed with a solution of p-nitrobenzenediazoniumfluoborate in acetone while the center of the plate is covered. The boundary of the adsorption layer of the phenol concentrations to be determined is characterized by the phenols made visible on both edges of the glass plate. These surfaces are scraped off and the substance placed on a frit (Germ. make "G4"). Here it is treated 6 times with 4 cm^3 of methanol and sucked into a 50 cm^3 measuring flask. To the methanolic solution of the phenol one then adds 2 cm^3 of a 10% watery sodium acetate solution and 2 cm^3 of a 0.1% watery p-nitrobenzenediazoniumfluorborate solution, which for stabilization contains 2% of a 35% HBF_4 solution. Then it is left to stand for 5 minutes. After this, 10 cm^3 of 1n KOH is added to this slightly colored solution, water is added up to the calibration mark, it is shaken well, and the testing is carried out immediately using a dark blue filter for phenol and a green filter for resorcinol and catechol.

We examined the quantitative determination of phenols that were separated by means of thin-layer chromatography using waste water samples of the Tar Processing Firm, Erkner, Germany. This water contained a mixture of monovalent phenols which we were not able to separate by using thin-layer chromatography. The usual bromide-bromate analysis yielded a total phenol of 7.06 g/ℓ. After applying the water directly to a thin-layer plate and conducting the analysis as previously described, we obtained in several tests a total phenol of about 7.5 g/ℓ.

After extracting the phenol-containing waste water three times with butyl acetate, we obtained, by using bromide-bromate, a total phenol of 0.56 g/ℓ. Several tests showed that direct application of this diluted phenolic water did not enable us to obtain a corresponding analysis of the values, and it was necessary to enrich the phenols. For this purpose, 500 cm^3 of water was acidified with phosphoric acid and extracted with ether in a Kutscher-Steudel device for 2 hours. After an extraction time of only 1½ hours, no phenols could be detected with 4-aminoantipyrine in the water. The solution was stripped from ether by distillation. The remaining butyl cetate was transferred to a 50 cm^3 measuring flask and filled with butylacetate. This solution was applied on the super gel plate and analyzed as described. It was in this way that we found 0.47 g/ℓ of monovalent phenols. The result was reproducible several times. It is to be assumed that this value is given preference over the one obtained with bromide-bromate. By the use of thin-layer chromatography there were also no multivalent phenols detectable in water that was enriched at a ratio of 1 : 10.

Thus, we were able to show that a quantitative evaluation of phenols in industrial waste waters is possible through separation by means of thin-layer chromatography. The method is fast and simple, but its application is limited. The qualitative analysis of phenols through thin-layer chromatography can be used advantageously on a broader scale and is recommended for analyzing the most important phenols contained in waste waters.

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